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(54) **Use of glycosaminoglycans in the treatment of diabetic nephropathy and diabetic neuropathy**

Verwendung von Glycosaminoglycanen zur Behandlung von diabetischer Nephropathie und diabetischer Neuropathie

Utilisation de glycosaminoglycanes dans le traitement de la néphropatie et neuropathie diabètique

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Description

The use of some glycosaminoglycans, and particularly of heparins, in anticoagulant and antithrombotic therapies is well known, while their use in the treatment of the collateral pathologies of diabetes, like diabetic nephropathy and diabetic neuropathy, is unknown.

Kanwar Y.S. et al., Sem. Nephrol., 5, 307, (1985) and Groggel G.C. et al., Kidney Int., 33, 517, (1988), recently produced evidence of the probable role of glycosaminoglycans in helping the integrity and the functioning of the renal cells.

Moreover, Canfield J.P. et al., Lab. Invest., 39, 505, (1978), previoulsy showed a decrease of glycosaminoglycans of membrane in many conditions of nephropathy, while Baggio B. et al., Nephron., 43, 187, (1986) showed this decrease through an increased urinary elimination of glycosaminoglycans in diabetic, non-albuminuric, patients. This increased excretion of glycosaminoglycans in diabetic nephropathies, shown also by Partasarathy N. et al., Diabetes, 31, 738, (1982), recently suggested to Gambaro G. et al., Metabolism, 38, 419, (1989), the possibility of resorting to the determination of the amount of glycosaminoglycans excreted by urinary route as an analytical method more reliable than the microalbuminuria in the recognition of the nephropathy of diabetic origin.

Lastly, Diamond J.R. et al., Renal Physiol., 9, 366, (1986) and Parkerson M.B. et al., J. Clin. Invest., 81, 69, (1988). showed in animals the potential protective effect of heparin and its derivatives in models of experimental nephropathy not related to diabetic nephropathy, like chronic nephrosis from aminoglycosides and renal pathologies resulting from the subtotal renal ablation in the rat.

"Current Status of prevention and treatment of diabetic complications, 1990, 567-569" and "The Journal of Diabetic Complications 5 (2-3), 92-94 (1991)" relate to the successful treatment of diabetic patients suffering from diabetic nephropathy by subcutaneous injection of sodium heparin. WO-A-90/00058 describes the usefulness of glycosaminoglycans, such as chondroitin sulfate and salts and/or hydrates thereof, in the treatment of diabetic microangiopathies. "Revista cubana de medicina 27 (7), 92-97 (1988)" concerns the satisfactory treatment of diabetic polyneuropathy using heparin. "Probl. Endocrinol. 24 (6), 23-30 (1978)" discloses that intravenous infusion of heparin and derivatives thereof decreased the blood sugar level in diabetic patients. "Vtr. Bol. XVI, (6), 52-58 (1977)" describes that heparinoids both decrease the blood sugar level and alter the blood lipid fraction level in diabetic patients. "The Merck Index, 1989, page 344, no. 2217: Chondroitin-sulfate" discloses that chondroitin sulfate and dermatan sulfate are used as antihyperlipoproteinemics.

Pharmacological studies that show a possible role of exogenous glycosaminoglycans administered for prevention or therapy of diabetic glomerulopathy and diabetic nephropathy do not exist yet.

Therefore the demonstration of the therapeutic use of some glycosaminoglycans, particularly of heparin derivatives obtained by depolymerization or by other chemical modifications like, for instance, treatment in basic medium and of low molecular weight dermatan sulfates, in the prevention and therapy of diabetic nephropathy, is the aim of the present invention.

The demonstration of the possible therapeutic use of some glycosaminoglycans in another pathology related to diabetes, exactly in that morphologic and functional alteration of the peripheral nervous system named diabetic neuropathy, is a further scope of the present invention.

Diabetic neuropathy is a disease that hits the nerves and the neurons of the peripheral nervous system of diabetic patients. This pathology is characterized by a progressive morphofunctional alteration of this system that starts with a reduced functioning of the nerves, noticeable by a lowered speed of conduction of the nervous impulse, and that gradually proceeds up to the degeneration of the nerves and the atrophy of the neurons. This event causes a gradual loss of the sensory capacities (pain, warmth etc.), a decrease of the muscolar strenght and a serious degeneration of the autonomic nervous system. This latter complication is surely the most frequent among the complications caused by diabetes; as a matter of fact about 70% - 80% of the diabetic patients suffers from gastrointestinal disorders caused by the bad functioning and the degeneration of the autonomic enteric system.

This complication is directly related to the degeneration of two intrinsic neuron factors of the intestinal wall. They are the system containing Met-Enkephalin and that containing Substance p. Met-Enkephalin controls the contraction of the sphincter between stomach and intestine and moreover is able to modulate the excitability of the other enteric neurons. Substance P is contracting, therefore it is one of the substances responsible for the intestinal motility.

Di Giulio A.M. et al., J. Neurosc. Res., 24, 355-61, (1989), recently demonstrated that these two neuron systems degenerate, with loss of Substance P and Met-Enkephalin in the intestinal zones of duodenum and jejunum, in the experimental diabetes caused by alloxan.

Any bibliography does not exist up to now related to a possible implication of endogenous glycosaminoglycans in setting up the diabetic neuropathy and moreover pharmacological studies that show a possible role of the exogenous glycosaminoglycans administered with a prophylactic or therapeutic purpose in the neuropathy of diabetic origin do not exist.

Aim of the present invention is, therefore, the demonstration of the therapeutic use of some glycosaminoglycans, particularly of heparin derivatives obtained by depolymerization or by other chemical modifications like, for instance,

treatment in basic medium and of low molecular weight dermatan sulfates in the prevention and therapy of diabetic neuropathy.

The object of the present invention is the manufacture of a medicine for the use of some glycosaminoglycans in the prevention and treatment of some diabetic pathologies that hit the renal system and the peripheral nervous system, particularly the pathologies known under the name of diabetic nephropathy and diabetic neuropathy.

The object of the present invention is solved by the use of glycosaminoglycans selected from low molecular weight heparins obtained by chemical depolymerization, heparin derivatives chemically modified in basic medium and low molecular weight dermatan sulfates obtained by chemical depolymerization in the manufacture of a medicine for the prevention and treatment of diabetic nephropathy and diabetic neuropathy.

The evaluation of the capability of these glycosaminoglycans to prevent and treat these pathologies has been carried out by means of pharmacological tests on male albino rats made diabetic by treatment with streptozotocin or alloxan. The measure of the glomerular anionic charges, the measure of the thickness of the basal glomerular membrane and the evaluation of albuminuria during 24 hours in rats made diabetic with streptozotocin in comparison with some diabetic rats treated with three different kinds of glycosaminoglycans and with non-diabetic rats, were the tests carried out for diabetic nephropathy. These tests were selected because the thickening of the basal glomerular membrane and the decrease of the glomerular anionic charges, outcome of a trouble of glycosaminoglycans metabolism, are elements that characterize the diabetic glomerulopathy and because such alterations can form the pathologic base of the proteinuric renal syndromes typical of the diabetic nephropathy determinable through the measure of albuminuria during 24 hours.

Parameters like the measure of the levels of Met-Enkephalin and Substance P in some intestinal zones, like duodenum and jejunum, of rats made diabetic with alloxan in comparison with the same diabetic rats treated with three different kinds of glycosaminoglycans and with the non-diabetic rats treated or not with the same three different kinds of glycosaminoglycans, were examined for the evaluation of the effectiveness of glycosaminoglycans towards diabetic neuropathy.

As a matter of fact, Substance P and Met-Enkephalin are an index of the degeneration of the enteric neurons caused by diabetic neuropathy produced by the experimental diabetes from alloxan. In fact Di Giulio A.M. et al., J. Neurosc. Res., 24, 355-61, (1989) showed an alteration of the gastroenteric innervation, which is a serious symptom of diabetic neuropathy, in the chronic experimental diabetes. Because of this alteration, the content of Substance P and Met-Enkephalin decreases significantly in many intestinal zones. The lowering of Substance P and Met-Enkephalin can be quantized and therefore the effect of the pharmacologic treatment can be determined with great accuracy, by means of a radioimmunoassay with specific antibodies described by Di Giulio A.M. et al., Brain Res., 342, 405-8, (1985).

The pharmacological tests for the determination of the therapeutic effectiveness of some glycosaminoglycans in the treatment of diabetic nephropathy and diabetic neuropathy, carried out on groups of male albino rats, are described in detail in two examples that illustrate the invention without limiting it. Said tests clearly show the achievement of the goal of the present invention because the values of the parameters related to the diabetic rats treated with these glycosaminoglycans correspond to the values of the non-diabetic control rats and therefore they are normal, while the values of the parameters related to the diabetic rats not treated with these glycosaminoglycans show significant differences that fully document the serious pathologic condition of the experimental animals.

In particular, the urinary excretion of albumin during 24 hours, albuminuria, is extremely higher in the diabetic rats not treated with these glycosaminoglycans, seven times higher on average in comparison with the control rats, while it does not show any statistically significant differences in the diabetic rats treated with these glycosaminoglycans. The thickness of the basal glomerular membrane analogously increases in statistically significant way and the density of the anionic charge decreases in statistically significant way in the untreated diabetic rats in comparison with the non-diabetic control rats, while no statistically significant difference is detectable among the control rats and the diabetic rats treated with these glyosaminoglycans.

Finally, the experimental data related to the contents of Substance P and Met-Enkephalin in the intestinal zone show a statistically significant decrease of Substance P and Met-Enkephalin in the duodenum and the jejunum of the untreated diabetic rats in comparison with the control rats, while the treatment with these glycosaminoglycans keeps statistically unchanged both the level of Substance P and that of Met-Enkephalin.

Low molecular weight heparins obtained by chemical depolymerization, heparins chemically modified in basic medium and low molecular weight dermatan sulfates obtained by chemical depolymerization can be advantageously used within the present invention.

The low molecular weight heparins obtained according to the method of chemical depolymerization described in European Patent EP 0121067, the heparin derivatives chemically modified in basic medium described in European Publication EP 0380943 and the low molecular weight dermatan sulfates obtained according to the method of the chemical depolymerization described in International Publication (PCT) WO 86/06729 are particularly preferred in the fulfillment of the present invention.

The low molecular weight heparins, having an average molecular weight equal to 4,500 ± 1,000 Daltons, are obtained, according to European Patent EP 0121067, by treating in aqueous solution commercial heparin with cupric acetate, hydrogen peroxide and ascorbic acid at a temperature of 40°-50°C and at a pH of 7.5-8.

The heparin derivatives chemically modified in basic medium are obtained, according to European Publication EP 0380943, by reacting an aqueous solution containing a commercial, purified or low molecular weight heparin, with a 0.01N-1N solution of an alkali or alkaline earth metal base for a period of time between 0.5 and 24 hours at a temperature between 75°C up to the boiling temperature of the reaction mixture. These heparin derivatives are characterized by a ^{13}C-NMR spectrum in the zone between 102 and 92 p.p.m. exhibiting a characteristic signal at 101.3 p.p.m., a specific rotatory power at 546 nm between +15° and +40° in aqueous solution, sulphur content between 6% and 9%, a sulfate/carboxyl ratio between 1.20 and 1.70 and a free amino group content between 0.4% and 2.1%.

The low molecular weight dermatan sulfates, having a molecular weight between 3,500 and 8,000 Daltons, are obtained, according to International Publication (PCT) WO 86/06729, by depolymerization of dermatan sulfate in aqueous solution in the presence of cupric acetate and hydrogen peroxide at a temperature between 20° and 50°C for a period of time between 1 and 2 hours.

Said glycosaminoglycans can be administered through medicines suitable both for the classic administration routes like the intramuscular or intravenous route and for other routes like those subcutaneous, transdermal, iontophoretic or oral in the prevention and treatment of diabetic nephropathy and diabetic neuropathy.

The glycosaminoglycans used in the two examples that illustrate the invention without limiting it are the low molecular weight heparin obtained according to the process of chemical depolymerization described in European Patent EP 0121067, having an average molecular weight equal to 4500 ± 1000 Daltons, the low molecular weight dermatan sulfate obtained according to the process of chemical depolymerization described in International Publication (PCT) WO 86/06729, having an average molecular weight equal to 5500 ± 1350 Daltons, and a heparin derivative chemically modified in basic medium obtained according to the process described in European Publication EP 0380943.

EXAMPLE 1

Experimental treatment of diabetic nephropathy with some glycosaminoglycans

Diabetes was induced by administering an aqueous physiologic solution containing 35 mg of streptozotocin for each kilogram of body weight by intravenous route to 12 male albino Sprague-Dawley rats 7 weeks old coming from the Charles River farm in Como. Three male albino Sprague-Dawley rats having the same age and origin, which were injected with a physiologic solution without streptozotocin, were the controls.

The treatment with streptozotocin caused diabetes in all the treated rats as demonstrated by glycosuria constantly higher than 1000 mg/dl. During the whole time of the experimentation, the animals were fed ad libitum by means of a standard diet, based on *Altromin*® of the firm Riefer of Bolzano, having a protein content equal to 20%. A week after induction of diabetes by means of streptozotocin, the 12 diabetic rats were divided into four groups of three:

GROUP A - Control diabetic rats;
GROUP B - Diabetic rats treated with low molecular weight heparin;
GROUP C - Diabetic rats treated with low molecular weight dermatan sulfate;
GROUP D - Diabetic rats treated with heparin derivative chemically modified in basic medium.

The three non-diabetic rats were the control GROUP E.

During a period of eight months, five days a week, the diabetic rats of GROUP B were treated with 6 mg/kg/die of low molecular weight heparin administered by subcutaneous route in 1 ml of physiologic solution, the diabetic rats of GROUP C with 15 mg/kg/die of low molecular weight dermatan sulfate and the diabetic rats of GROUP D with 15 mg/kg/die of heparin derivative chemically modified in basic medium, according to the same manner. A physiologic solution without any active principle was contemporaneously administered, under cutis, with the same procedure, to the diabetic rats of GROUP A and to the control rats of GROUP E.

The weight of the animals was controlled once a month and the rats were stabled during 24 hours in single cages for the determination of the diuresis.

During this period of eight months, all the diabetic rats turned out to be under weight in comparison with the healthy controls and showed strong hyperglycemia, polyuria, glycosuria and acidic urines together with ketonuria so showing the dismetabolic condition.

Eight months after induction of diabetes, the animals were submitted to the 24 hours diuresis for the determination of albuminuria and then were submitted to laparatomy.

The abdominal aorta was isolated, a suture thread was passed under it by way of a slip-knot over the renal arteries and then a second thread was passed underneath. A small catheter made of polyethylene was introduced into the aorta under the second slip-knot pushing it up to the level of the renal artery where it was fixed by clasping the lower slip-knot.

Subsequently, after having cut the renal veins and clasped the upper slip-knot, a solution containing 0.2% of red ruthenium in Karnowski's fixative was continuously instilled by means of a pump, under a pressure of 100 mm of mercury.

Afterwards the kidney was taken out and the renal capsule was unfolded and the parenchyma was opened as a page of a book up to the pelvis by means of a lengthwise cut along the great curve. Two fragments having a side not less than 1÷2 mm were taken from the cortex and were soaked for 20 hours at room temperature in the Karnowski's fixative containing 0.2% of red ruthenium. Subsequently, the cortex' takings were twice washed with cacodylate buffer and then submitted to the post-fixation treatment for one hour at room temperature in aqueous solution containing 1% of osmium tetroxyde and 0.05% of red ruthenium. Afterwards the takings were dehydrated first putting them in ethyl alcohol and then in propylene oxide. The so obtained small blocks of tissue were embedded in Epon and cut with the ultramicrotome for examining through the electronic microscopy the thickness of the basal glomerular membrane and the number of anionic charges displayed by the treatment with red ruthenium.

Photographies at 35,000 enlargements were carried out for the morphometric evaluation and the mean thickness of the basal glomerular membrane was measured.

By using a semi-automatic image analyzer Ibas Kontron, the lenght of the external surface of the basal glomerular membrane was measured and the number of the anionic charges displayed by the treatment with red ruthenium was counted, relating said number to 1000 nm. The statistical evaluation of the means among the groups of rats was carried out with the non-parametric test of Wilcoxon F., described in "Individual comparison by ranking methods", Biometr. Bull., 1, 80-3, (1945), at the significance threshold p=0.05.

The experimental data related to the thickness of the basal membrane, to the number of the glomerular anionic charges and to albuminuria are reported in the following table 1.

TABLE 1

GROUPS OF RATS	Glomerular anionic charges (number/1000 nm) $\bar{X} \pm$ s.e.	Thickness of the basal membrane (nm) $\bar{X} \pm$ s.e.	Albuminuria (µg/die) $\bar{X} \pm$ s.e.
A	22.17 ± 4.20	375 ± 127	318.67 ± 31.34
B	43.43 ± 3.25	238 ± 39.89	21.20 ± 5.21
C	36.47 ± 6.54	265 ± 21.94	56.67 ± 36.65
D	40.97 ± 5.18	221 ± 33.15	30.56 ± 9.77
E	38.53 ± 1.61	235 ± 15.89	45.63 ± 25.42

The experimental data reported in table 1 clearly show that the diabetic rats not treated with glycosaminoglycans (GROUP A) undergo serious morphologic anomalies, strong thickening of the basal membrane and remarkable decrease of the glomerular anionic charges, which, consequently, go with a very evident clinical anomaly shown by values of albuminuria even seven times higher than those of the control rats (GROUP E).

On the contrary, both the diabetic rats treated with low molecular weight heparin (GROUP B), the diabetic rats treated with low molecular weight dermatan sulfate (GROUP C) and the diabetic rats treated with a heparin derivative chemically modified in basic medium (GROUP D), show values both of the morphologic parameters and of the clinical parameter in agreement with the data of the group of the non-diabetic control rats (GROUP E).

Therefore the treatment with glycosaminoglycans like the low molecular weight heparin, the low molecular weight dermatan sulfate and the heparin derivative chemically modified in basic medium, is able to cause remarkable morphologic and clinic improvements in the experimental model of diabetic nephropathy caused by streptozotocin in the rat. As a matter of fact, the capability of glycosaminoglycans to prevent diabetic nephropathy is clearly demonstrated by the lack of thickening of the basal glomerular membranes and of the decrease of the glomerular anionic charges and by the inhibition of the appearance of albuminuria.

EXAMPLE 2

Experimental treatment of diabetic neuropathy with some glycosaminoglycans

Diabetes was induced by means of a subcutaneous injection of alloxan dissolved in a pH 3 citrate-phosphate buffer at the dosage of 100 mg for each kilogram of body weight, in male albino Sprague-Dawley rats weighing 250 g coming from the Charles River farm in Como. The appearance of diabetes was ascertained a week after the treatment with alloxan by determining glycosuria with the Glucur Test of Boehringer Biochemia and the glycemia by means of the hexokinase method with the Gluco-Quant of Boehringer Biochemia. Only the diabetic rats having a value of glycemia

higher than 400 mg/dl and a body weight lower than 320 g were used for the experimentation. Male albino Sprague-Dawley rats having the same age and weight and treated with a subcutaneous injection of citrate-phosphate buffer alone were used as non-diabetic control rats.

Both diabetic and control rats were stabled and fed in the same manner, with water and food ad libitum.

The diabetic rats were divided into four groups of 12:

GROUP A - Control diabetic rats;
GROUP B - Diabetic rats treated with low molecular weight heparin;
GROUP C - Diabetic rats treated with low molecular weight dermatan sulfate;
GROUP D - Diabetic rats treated with a heparin derivative chemically modified in basic medium.

Also the non-diabetic control rats were divided into four groups of 12:

GROUP E - Non-diabetic control rats;
GROUP F - Non-diabetic rats treated with low molecular weight heparin;
GROUP G - Non-diabetic rats treated with low molecular weight dermatan sulfate;
GROUP H - Non-diabetic rats treated with heparin derivative chemically modified in basic medium.

During a period of time of 18 weeks, groups B and F were treated with 6 mg/kg/die of low molecular weight heparin administered by subcutaneous route in 1 ml of physiologic solution, groups C and G with 15 mg/kg/die of low molecular weight dermatan sulfate and groups D and H with 15 mg/kg/die of heparin derivative chemically modified in basic medium, in the same manner, for 5 days a week, starting from the week following that of the induction of diabetes with alloxan. A physiological solution without any active principle was contemporaneously administered under cutis in the same manner to the control diabetic rats of group A and to the control non-diabetic rats of group E.

All the animals were fasted for 24 hours and then were killed by decapitation at the end of the 18 weeks of treatment. The intestine was dissected in segments; the duodenum and the jejunum were isolated, carefully washed with Krebs solution cooled to about 0°C and lastly dissected in 5mm long pieces.

These specimens were frozen at -30° C with liquid nitrogen till the moment of carrying out the radioimmunoassays for the determination of Substance P and Met-Enkephalin that were determined by using specific immunizing sera as described by Di Giulio A.M. et al., Brain Res., 342, 405-8, (1985).

The experimental data, expressed as mean ($\bar{X}$) ± standard error (s.e.), related to the levels of Substance P and Met-Enkephalin, measured as ng/mg protein, in the duodenum and the jejunum of the experimental animals, are reported in the following tables 2 and 3.

TABLE 2

LEVELS OF SUBSTANCE P		
GROUPS OF RATS	Amount of Substance P in the duodenum (ng/mg protein) $\bar{X}$ ± s.e.	Amount of Substance P in the jejunum (ng/mg protein) $\bar{X}$ ± s.e.
A	0.28 ± 0.024	0.30 ± 0.018
B	0.41 ± 0.021	0.38 ± 0.016
C	0.38 ± 0.060	0.51 ± 0.018
D	0.43 ± 0.019	0.44 ± 0.028
E	0.40 ± 0.025	0.37 ± 0.023
F	0.42 ± 0.011	0.36 ± 0.022
G	0.33 ± 0.025	0.36 ± 0.014
H	0.39 ± 0.045	0.41 ± 0.036

TABLE 3

LEVELS OF MET-ENKEPHALIN		
GROUPS OF RATS	Amount of Met-Enkephalin in the duodenum (ng/mg protein) $\overline{X} \pm$ s.e.	Amount of Met-Enkephalin in the jejunum (ng/mg protein) $\overline{X} \pm$ s.e.
A	0.037 ± 0.037	0.50 ± 0.041
B	0.14 ± 0.009	0.88 ± 0.021
C	0.09 ± 0.018	0.80 ± 0.038
D	0.15 ± 0.021	0.85 ± 0.033
E	0.19 ± 0.013	0.90 ± 0.031
F	0.17 ± 0.015	0.93 ± 0.022
G	0.18 ± 0.009	0.80 ± 0.028
H	0.16 ± 0.008	0.87 ± 0.043

The experimental data clearly show that the levels (in ng/mg protein) of both Substance P and Met-Enkephalin in the duodenum and the jejunum of the untreated diabetic animals (group A) are significantly lower than those found in the corresponding organs of the healthy control animals (group E). This decrease of Substance P and Met-Enkephalin is prevented by the pharmacological treatments with glycosaminoglycans (groups B, C and D). As a matter of fact, the data of the groups B, C and D do not show any significant difference in comparison with the control group E. Moreover, the results obtained with the groups F, G and H, i.e. with the non-diabetic animals treated with glycosaminoglycans, clearly show that the administration of these glycosaminoglycans does not significantly change the content of substance P and Met-Enkephalin in the healthy animals. This fact shows that the pharmacological effect of these glycosaminoglycans occurs only on the cause of the neuropathy and not on the synthesis of Substance P and Met-Enkephalin in the healthy animals.

Moreover, the effect of normalization by these glycosaminoglycans on substance P and Met-Enkephalin in the diabetic animals goes with a concomitant maintenance of levels of glycemia equal to those of the untreated diabetic rats. Therefore all these experimental data show that these glycosaminoglycans are active in the inhibition of the experimental diabetic neuropathy, a fact that justifies the claim of the use of these glycosaminoglycans in the prevention and treatment of diabetic neuropathy.

Claims

1. Use of glycosaminoglycans selected from low molecular weight heparins obtained by chemical depolymerization, heparin derivatives chemically modified in basic medium and low molecular weight dermatan sulfates obtained by chemical depolymerization in the manufacture of a medicine for the prevention and treatment of diabetic nephropathy and diabetic neuropathy.

2. Use according to claim 1 characterized in that the low molecular weight heparins are obtained from commercial heparin in the presence of cupric acetate, hydrogen peroxide and ascorbic acid at a temperature of 40° - 50°C and at a pH of 7.5 - 8.

3. Use according to claim 1 characterized in that the heparin derivatives chemically modified in basic medium have a ^{13}C-NMR spectrum in the zone between 102 and 92 ppm exhibiting a characteristic signal at 101.3 ppm, a specific rotatory power at 546 nm between +15° and +40° in aqueous solution, a sulphur content between 6% and 9%, a sulfate/carboxyl ratio between 1.20 and 1.70 and a free amino group content between 0.4% and 2.1% and are prepared by reacting an aqueous solution containing a commerical, purified or low molecular weight heparin, with a 0.01 N - 1 N solution of an alkali or alkaline earth metal base for a period of time between 0.5 and 24 hours at a temperature between 75°C up to the boiling temperature of the reaction mixture.

4. Use according to claim 1 characterized in that the low molecular weight dermatan sulfates are obtained by depolymerization of dermatan sulfate in aqueous solution at temperatures between 20°C and 70°C by a radicalic reac-

tion initiated by the $^{\bullet}OH$ radical generated from hydrogen peroxide in the presence of a catalyst consisting of a metal such as Cu^{++}, Fe^{++} or Cr^{+++}.

Patentansprüche

1. Verwendung von Glycosaminoglycanen, ausgewählt aus Heparinen mit niedrigem Molekulargewicht, erhalten durch chemische Depolymerisation in einem basischen Medium, chemisch modifizierten Heparinderivaten und Dermatansulfaten mit niedrigem Molekulargewicht, erhalten durch chemische Depolymerisation, zur Herstellung eines Medikaments zur Verhütung und Behandlung von diabetischer Nephropathie und diabetischer Neuropathie.

2. Verwendung nach Anspruch 1, dadurch **gekennzeichnet,** daß die Heparine mit niedrigem Molekulargewicht aus handelsüblichem Heparin, in Gegenwart von Kupfer(II)-acetat, Wasserstoffperoxid und Ascorbinsäure bei einer Temperatur von 40° bis 50°C und einem pH-Wert von 7,5 bis 8 erhalten werden.

3. Verwendung nach Anspruch 1, dadurch **gekennzeichnet,** daß die im basischen Medium chemisch modifizierten Heparinderivate ein ^{13}C-NMR-Spektrum in der Zone zwischen 102 und 92 ppm mit einem charakteristischen Signal bei 101,3 ppm, ein spezifisches Drehvermögen bei 546 nm zwischen +15° und +40°C in wäßriger Lösung, einen Schwefelgehalt zwischen 6% und 9%, ein Sulfat/Carboxyl-Verhältnis zwischen 1,20 und 1,70 und einen freien Aminogruppengehalt zwischen 0,4% und 2,1% besitzen und durch Umsetzen einer wäßrigen Lösung, die ein im Handel erhältliches gereinigtes oder niedrigmolekulares Heparin enthält, mit einer 0,01 N bis 1 N Lösung einer Alkali-oder Erdalkalibase für eine Zeitspanne zwischen 0,5 und 24 Stunden bei einer Temperatur zwischen 75°C bis zu der Siedetemperatur des Reaktionsgemisches, hergestellt worden sind.

4. Verwendung nach Anspruch 1, dadurch **gekennzeichnet,** daß die Dermatansulfate mit niedrigem Molekulargewicht durch Depolymerisation von Dermatansulfat in wäßriger Lösung bei Temperaturen zwischen 20°C und 70°C durch eine radikalische Reaktion, die durch das aus Wasserstoffperoxid in Gegenwart eines Katalysators, bestehend aus einem Metall wie Cu^{++}, Fe^{++} oder Cr^{+++}, erzeugte $^{\bullet}OH$-Radikal gestartet wurde, erhalten worden sind.

Revendications

1. Utilisation de glycosaminoglycanes choisies parmi des héparines de bas poids moléculaire obtenues par dépolymérisation chimique, des dérivés d'héparine modifiés chimiquement dans un milieu basique et des dermatan sulfates de bas poids moléculaire obtenus par dépolymérisation chimique, dans la fabrication d'un médicament pour la prévention et le traitement de néphropatie diabétique et de neuropathie diabétique.

2. Utilisation suivant la revendication 1, caractérisée en ce que les héparines de bas poids moléculaire sont obtenues à partir d'héparine du commerce en présence d'acétate cuivrique, de peroxyde d'hydrogène et d'acide ascorbique à une température comprise entre 40°C et 50°C et à un pH de 7,5 à 8.

3. Utilisation suivant la revendication 1, caractérisée en ce que les dérivés d'héparine modifiés chimiquement dans un milieu basique ont un spectre RMN de ^{13}C dans la zone allant de 102 à 92 ppm présentant un signal caractéristique à 101,3 ppm, un pouvoir rotatoire spécifique à 546 nm compris entre +15°C et 40°C en solution aqueuse, une teneur en soufre comprise entre 6% et 9%, un rapport sulfate/carboxy compris entre 1,20 et 1,70 et une teneur en groupe amino libre comprise entre 0,4% et 2,1% et sont préparés par réaction d'une solution aqueuse contenant une héparine du commerce purifiée ou de bas poids moléculaire avec une solution 0,01 N à 1 N d'une base de métal alcalin ou d'une base de métal alcalino-terreux pendant une période de temps comprise entre 0,5 et 24 heures à une température comprise entre 75°C et le point d'ébullition du mélange réactionnel.

4. Utilisation suivant la revendication 1, caractérisée en ce que les dermatan sulfates de bas poids moléculaire sont obtenus par dépolymérisation de dermantan sulfates en solution aqueuse à des températures comprises entre 20°C et 70°C par une réaction radicalaire initiée par le radical $^{\bullet}OH$ générée par le peroxyde d'hydrogène en présence d'un catalyseur consistant en un métal tel que Cu^{++}, Fe^{++} ou Cr^{+++}.